



Concave microwell based size-controllable hepatosphere as a three-dimensional liver tissue model

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ABSTRACT

We have developed a size-controllable spheroidal hepatosphere and heterosphere model by mono-culturing of primary hepatocytes and by co-culturing primary hepatocytes and hepatic stellate cells (HSCs). We demonstrated that uniform-sized heterospheres, which self-aggregated from primary hepatocytes and HSCs, formed within concave microwell arrays in a rapid and homogeneous manner. The effect of HSCs was quantitatively and qualitatively investigated during spheroid formation, and HSC played an important role in controlling the organization of the spheroidal aggregates and formation of tight cell–cell contacts. An analysis of the metabolic function showed that heterospheres secreted 30% more albumin than hepatospheres on day 8. In contrast, the urea secretion from heterospheres was similar to that of hepatospheres. A quantitative cytochrome P450 assay showed that the enzymatic activity of heterospheres cultured for 9 days was higher as compared with primary hepatospheres. These size-controllable heterospheres could be mass-produced using concave plate and be useful for creating artificial three-dimensional hepatic tissue constructs and regeneration of failed liver.

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1. Introduction

The liver consists of primary hepatocytes, hepatic stellate cells (HSCs), Kupffer cells, endothelial cells, and fibroblasts [1,2]. Hepatocytes, which constitute 70–80% of the liver cytoplasmic mass, are central to liver physiology and function, playing an important role in the secretion of plasma proteins and the oxidative metabolism of drugs and xenobiotics [3–5]. They are also targets of pathophysiological stimuli, including cytokines [4], growth factors [4,6–8] and hormones [4], that influence liver-specific functions [7,9].

Although two-dimensional (2D) monolayer cultures of primary hepatocytes have been extensively employed as an *in vitro* model for pharmacological, toxicological, and metabolic studies, the rapid loss of hepatocyte metabolic functions has been a critical drawback [10–15]. In contrast, three-dimensional (3D) cultures of hepatocytes, termed hepatospheres, maintain liver-specific functions because of their tissue-like structure, showing that hepatosphere represent a robust model for investigating metabolic functions [16–22]. Hepatospheres enable the control of several liver-specific functions, including albumin secretion, urea synthesis, drug

metabolism, and bile acid metabolism. Hepatospheres also exhibit higher cadherin and lower integrin expression than monolayer single cells, suggesting that they enhance cell–cell contact [16,23–25]. Given that hepatospheres have tight cell–cell contacts, they might suggest a potential solution for a therapeutic modality in repairing and regulating damaged or diseased liver tissues.

To date, several approaches for producing hepatospheres have been previously reported, including non-adherent surface [26,27], matrix array [7,28], and rocked culture [18,29]. However, the development of well-controlled 3D culture systems capable of producing large amounts of uniform hepatospheres has remained an unmet challenge, such as size control of hepatosphere, because necrosis may occur inside the core of large spheroids [27]. One method for addressing this limitation is the development of micropatterns of spheroids using microscale technologies, such as micromolding techniques for cell-adhesive microarray patterns [28]. Another method for regulating spheroid formation is microwell arrays, which can control cell docking and immobilization on non-cell-adhesive surfaces [30]. Although these previous approaches can create spheroids, they are not spherical shape and additional processes are required to harvest the cells. An additional challenge is the development of a spheroidal co-culture model, heterospheres consisting of hepatocytes and HSCs that can mimic *in vivo* extracellular microenvironment. In spite of extensive work

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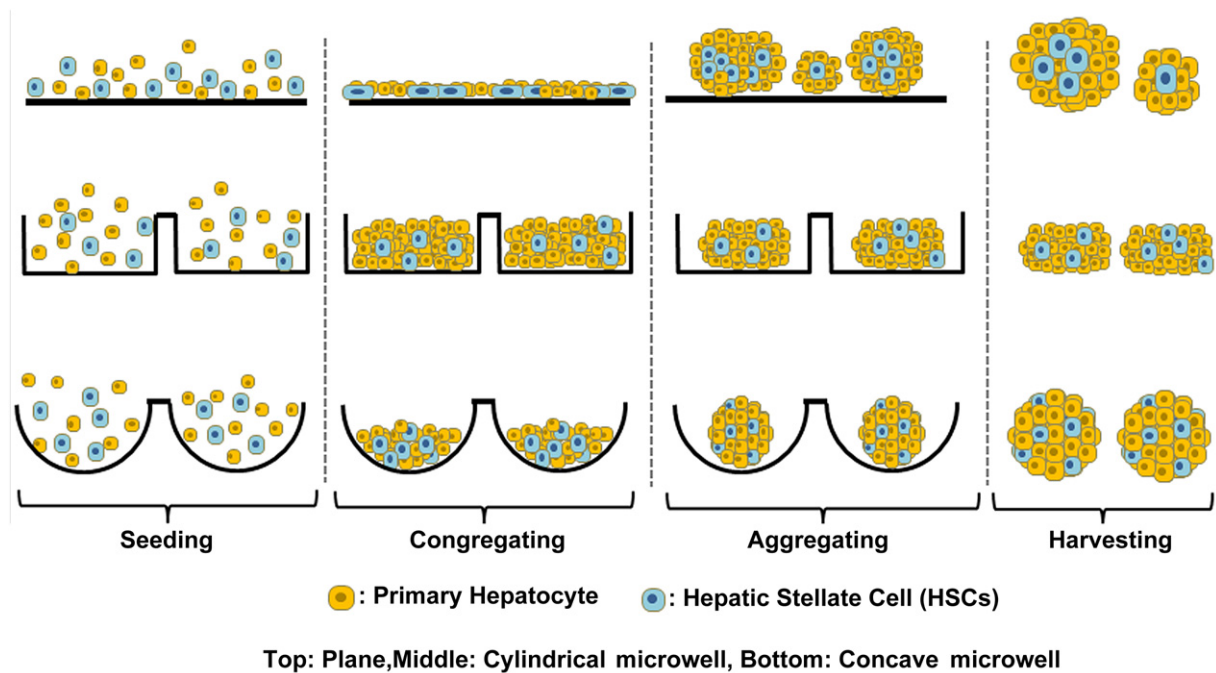


Fig. 1. Schematic illustration of the co-culture models of hepatospheres (primary hepatocytes only) and heterospheres (3:1 ratio of primary hepatocytes and HSCs) according to the substrate: (top) plane surface, (middle) cylindrical microwell, (bottom) concave microwell.

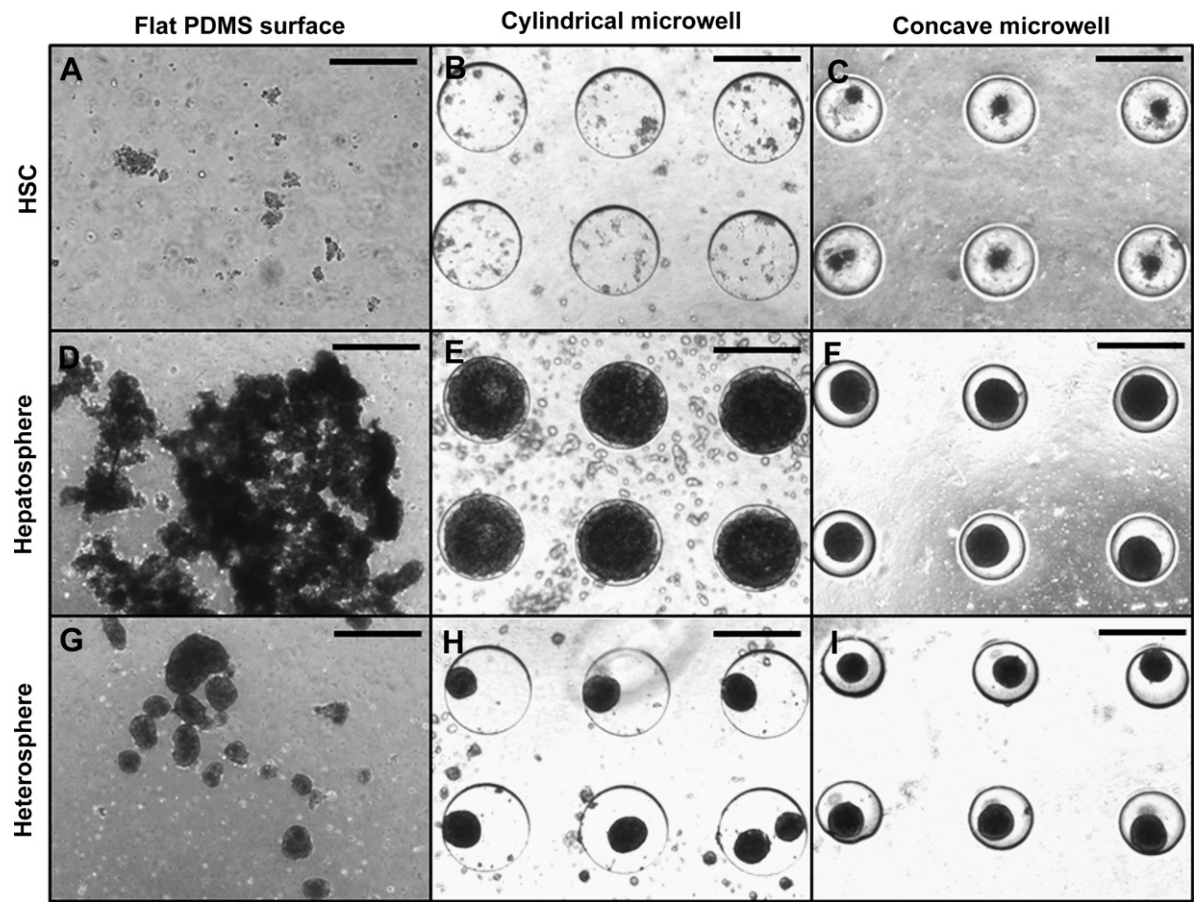


Fig. 2. (A–C) HSCs cultured on the plane surface, cylindrical microwell and concave microwell, and HSCs are aggregate only in concave microwell; (D–F) hepatocytes cultured on the plane surface, cylindrical microwell and concave microwell; and (G–I) mixture of hepatocytes and HSC heterospheres cultured on the plane surface, cylindrical microwell and concave microwell, and multiple spheroids are observed in cylindrical microwell. Scale bars, 50 μ m.

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